

**Clean Copy of Amended Claims**

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1 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

A<sup>1</sup>  
incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

2 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

3 (amended). The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

4 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;

(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

*A1*  
*coind*

5 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

*A2*

6 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

*A2*

9 (amended. The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

*A3*

12 (amended). The method of claim 1, wherein said selection is performed by culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium; contacting said cells with a selection agent; and selecting transformed cells.

*A4*

15 (amended). The method of claim 14, wherein said layer is a layer of liquid medium.

16 (amended). The method of claim 14, wherein said layer is a layer of gelled medium.

*A5*

19 (amended). The method of claim 1 which further comprises the eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

20 (amended). The method of claim 19, wherein said eradication is performed by: culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and recovering cells from which said *Agrobacterium* has been eradicated.

*A5  
compl'd*

21 (amended). The method of claim 20, wherein said layer is a layer of liquid medium.

22 (amended). The method of claim 20, wherein said layer is a layer of gelled medium.

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25 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

*A4*  
incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

26 (amended). The method of claim 25, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;  
(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

27 (amended). The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

28 (amended). The method of claim 26, wherein said selection is performed by culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and  
selecting transformed cells.

29 (amended). The method of claim 26, wherein said eradication is performed by:  
culturing cells which have been incubated with *Agrobacterium* on a support membrane over  
a layer containing an eradicant, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.

*A6  
cont'd*

30 (amended). The method of claim 28, wherein said eradication is performed by:  
culturing cells which have been incubated with *Agrobacterium* on a support membrane over  
a layer containing an eradicant, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.

31 (amended). The method of claim 25, wherein said damage to cells is minimized by:  
(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;  
(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and  
(c) recovering washed cells with minimal damage.

32 (amended). The method of claim 31, wherein pine cells are plated onto a support  
membrane prior to *Agrobacterium* transformation.

33 (amended). The method of claim 31, wherein pine cells are plated onto a support  
membrane subsequent to *Agrobacterium* transformation.

34 (amended). The method of claim 31, wherein said selection is performed by  
culturing cells which have been incubated with *Agrobacterium* on a support membrane  
placed over a gel medium;

contacting said cells with a selection agent; and  
selecting transformed cells.

35 (amended). The method of claim 31, wherein said eradication is performed by:  
culturing cells which have been incubated with *Agrobacterium* on a support membrane over  
a layer containing an eradicant, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.

*A 4  
cont'd*

36 (amended). The method of claim 34, wherein said eradication is performed by:  
culturing cells which have been incubated with *Agrobacterium* on a support membrane over  
a layer containing an eradicant, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.

37 (amended). The method of claim 25, wherein said selection is performed by  
culturing cells which have been incubated with *Agrobacterium* on a support membrane  
placed over a gel medium;  
contacting said cells with a selection agent; and  
selecting transformed cells.

38 (amended). The method of claim 25, wherein said eradication is performed by:  
culturing cells which have been incubated with *Agrobacterium* on a support membrane over  
a layer containing an eradicant, said layer in or positioned over a gel medium; and  
recovering cells from which said *Agrobacterium* has been eradicated.

39 (amended). A method for minimizing damage to transformed cells of pine of the genus  
*Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation  
which comprises:

(a) washing transformed cells of the subgenus *Pinus* in a liquid wash medium;

*A<sup>4</sup>  
cont'd*

- (b) plating said cells on a support membrane;
- (c) suspending said cells in a liquid wash medium; and
- (d) recovering washed cells with minimal physical damage.

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*A<sup>7</sup>*

43 (amended). The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

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*A<sup>8</sup>*

46 (amended). A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

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47 (amended). The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents, said layer is positioned on said gel medium.

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*A<sup>9</sup>*

49 (amended). The method of claim 47, wherein said layer is a layer of liquid medium.

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*A<sup>10</sup>*

52 (amended). A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus subsequent to transformation on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

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*A<sup>11</sup>*

55 (amended). The method of claim 54, wherein said layer is a layer of liquid medium.

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*A<sup>12</sup>*

58 (amended). A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an  
eradicant, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

*A12*  
*cont'd*  
59 (amended). The method of claim 58, wherein said layer is a layer of liquid medium.

60 (amended). The method of claim 58, wherein said layer is a layer of gelled medium.

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